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16. ABSTRACT

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The Reproductive Effects Assessment Group's Report on the Mutagenicity of 1,3-Butadiene and Its Reactive Metabolites

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A major data gap for assessing heritable risk from exposure to 1,3-butadiene is the lack of mammalian mutagenicity data. The data base on the mutagenic potential of 1,3-butadiene is limited to three bacterial studies from the same laboratory. Two of these studies were positive only in the presence of liver S9 mix from chemically pretreated animals. In vitro data suggest that 1,3-butadiene is metabolized to two epoxide intermediates. 3,4-Epoxybutene, one potential reactive metabolite of 1,3-butadiene, is a monofunctional alkylating agent and is a direct-acting mutagen in bacteria. In addition, unpublished data suggest that 3,4-epoxybutene induces DNA damage and chromosomal aberrations in mice. Another potential reactive metabolite, 1,2:3,4-diepoxybutane, is a bifunctional alkylating agent and is mutagenic in a wide variety of organisms (bacteria, fungi, and the germ cells of *Drosophila*). This metabolite also induces DNA damage in mice and in cultured hamster cells, is clastogenic in fungi and cultured rat cells, and produces chromosome damage/breakage in *Drosophila* germ cells. These data, when combined with evidence that 1,3-butadiene is carcinogenic in rodent gonadal tissues and is associated with gonadal atrophy in mice, constitute suggestive evidence that 1,3-butadiene may be a human germ cell mutagen. However, because the mutagenicity of 1,3-butadiene has been studied only in bacteria, studies in mammalian test systems are needed to further characterize the mutagenic potential of 1,3-butadiene.

Key words: mutagenesis, 1,3-butadiene, 3,4-epoxybutene, 1,2:3,4-diepoxybutane, reactive metabolites

INTRODUCTION

1,3-Butadiene, a colorless gas with a mild aromatic odor at standard temperature and pressure, is used primarily in the manufacture of synthetic rubber (styrene-butadiene rubber and polybutadiene rubber), most of which is used in the automobile tire industry [Kirk-Othmer, 1979]. Approximately 2.5 billion pounds of 1,3-butadiene

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was produced in the United States in 1984, making it among the top 20 organic chemicals manufactured in the United States [Chemical Engineering News, 1984]. The current permissible worker exposure limit adopted by the Occupational Safety and Health Administration [1982] in 1971 is 1,000 ppm as an 8-hr time-weighted average. This standard was based on acute effects such as irritation and drowsiness. However, 1,3-butadiene has subsequently been found to cause cancer in laboratory animals [Hazelton Laboratories Europe, Ltd., 1981; Huff et al, 1985]. For this reason, the American Conference of Governmental Industrial Hygienists [1984] has recently suggested an 8-hr time-weighted average of 10 ppm. The National Institute for Occupational Safety and Health [1984], using data collected in 1972–1974, has estimated that approximately 65,000 workers are potentially exposed to 1,3-butadiene. More recently, the Office of Toxic Substances of the US Environmental Protection Agency [1985] has estimated that up to 10,000 workers are exposed to 1,3-butadiene (primarily via inhalation) mostly in plants manufacturing 1,3-butadiene or using it to produce polymers such as synthetic rubber.

This report is concerned with the mutagenicity of 1,3-butadiene and also includes a discussion of the metabolism of 1,3-butadiene and the mutagenicity of its potential reactive metabolites (3,4-epoxybutene and 1,2:3,4-diepoxybutane). The available evidence suggests that 1,3-butadiene is mutagenic by virtue of its capacity to be metabolized to mutagenic intermediates.

MUTAGENICITY OF 1,3-BUTADIENE

The mutagenic potential of 1,3-butadiene was tested in the *Salmonella typhimurium* histidine reversion assay by de Meester et al [1978]. *Salmonella* strains TA1530, TA1535, TA1537, TA1538, TA98, and TA100 were exposed to 1,3-butadiene vapors for 20 hr in closed desiccators. Mutagenic activity was observed in strains TA1530 and TA1535 both in the presence and absence of S9 prepared from Aroclor-pretreated rats. The bacteria were exposed to only one dose of 1,3-butadiene and that dose was not clearly specified. This study suggests that 1,3-butadiene is a direct-acting base-pair-substitution mutagen in bacteria.

In a subsequent study, de Meester et al [1980] exposed strain TA1530 to 1,3-butadiene vapors for 24 hr at 0, 4, 8, 16, 24, and 32% (vol/vol) in closed desiccators. In the absence of S9 mix or in the presence of S9 prepared from untreated rats, no increase in the revertant frequency was observed. However, when the bacteria were exposed to 1,3-butadiene in the presence of S9 mix prepared from phenobarbitone- or Aroclor 1254-pretreated rats, mutagenic activity was observed. The number of histidine revertants increased in a dose-related fashion from 17 per plate in the absence of 1,3-butadiene up to 255 per plate at 16% (vol/vol) 1,3-butadiene. These results suggest that 1,3-butadiene itself is not a mutagen, but that it is metabolized to mutagenic intermediates that cause base-pair substitutions.

Data suggesting that the mutagenic metabolites are volatile were also reported by de Meester et al [1980]. Plates containing *Salmonella* but no S9 mix were coincubated in 1,3-butadiene atmospheres (4–32%) with plates containing *Salmonella* plus S9 mix from chemically pretreated animals. The reversion rates were enhanced in both sets of plates, indicating that 1,3-butadiene was metabolized in the plates containing S9 mix to volatile mutagens which were active in the plates without S9

mix. The number of mutant colonies was proportional to the level of 1,3-butadiene in the desiccators up to 16%.

This second study by de Meester and co-workers [de Meester et al, 1980] contradicts their earlier observation [de Meester et al, 1978] that 1,3-butadiene is mutagenic in the absence of S9 mix. If, in the earlier study, plates containing bacteria plus S9 mix were incubated in the same desiccators with plates containing bacteria but no S9 mix, volatile mutagenic metabolites of 1,3-butadiene generated in the first set of plates may have been responsible for the mutagenic effects observed in the second set of plates.

A study by Poncelet et al [1980] supports the conclusion of de Meester et al [1980] on the mutagenic potential of 1,3-butadiene in *Salmonella* strain TA1530. Mutagenic effects were observed when the assays were performed in a 16% gaseous atmosphere of 1,3-butadiene in the presence of Aroclor-induced S9 mix. When the bacteria were exposed to 1,3-butadiene under the conditions of the plate incorporation method or preincubation in liquid medium, mutagenicity was not observed.

In summary, the weight of the available evidence suggests that 1,3-butadiene is a promutagen in bacteria; its mutagenicity depends on metabolic activation by S9 mix prepared from chemically induced animals. No whole-animal mutagenicity studies have been reported.

METABOLISM OF 1,3-BUTADIENE AND ALKYLATION BY METABOLITES

As described in the previous section, 1,3-butadiene itself does not appear to be mutagenic. Mutagenic activity is observed only when 1,3-butadiene has been metabolized to reactive intermediates. A scheme for the metabolism of 1,3-butadiene, and the alkylating ability of the probable metabolites are briefly discussed in this section.

Metabolism

Malvoisin et al [1979] and Malvoisin and Roberfroid [1982] studied the metabolism of 1,3-butadiene in vitro using rat liver microsomes. They reported that the metabolism proceeds via a mixed-function oxidase-catalyzed oxidation to 3,4-epoxybutene (butadiene monoxide), and they suggest that this compound is subsequently metabolized to 1,2:3,4-diepoxybutane (diepoxybutane) and 3-butene-1,2-diol (Fig. 1). Both 3,4-epoxybutene and diepoxybutane are probably reactive intermediates, whereas 3-butene-1,2-diol and its metabolite 3,4-epoxy-1,2-butanediol are probably detoxification products. Preliminary evidence in rats suggest that 1,3-butadiene is metabolized to 3,4-epoxybutene in vivo [Bolt et al, 1983], indicating that the metabolic pathway outlined in Figure 1 on the basis of in vitro data may occur in vivo. Both 3,4-epoxybutene and diepoxybutane are mutagenic, as described more fully below. Malvoisin and Roberfroid [1982] state that their unpublished results indicate that 3-butene-1,2-diol and 3,4-epoxy-1,2-butanediol are not mutagenic.

Alkylation

The alkylating activity of the two potential reactive metabolites of 1,3-butadiene (3,4-epoxybutene and diepoxybutane) has been investigated, 3,4-epoxybutene in two studies and diepoxybutane in one study. Hemminki et al [1980] found that 3,4-epoxybutene alkylated 4-(p-nitrobenzyl)-pyridine (NBP) and deoxyguanosine, which are nucleophiles that were used as models for DNA. In both cases, aliquots of the

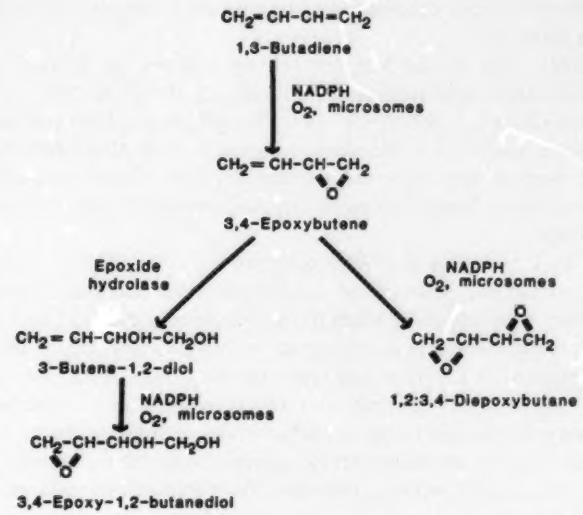

Fig. 1. A hypothetical scheme for the metabolism of 1,3-butadiene. Malvoisin and Roberfroid [1982] determined this metabolic scheme from in vitro experiments with rat liver microsomes.

reaction mixture were assayed for alkylation at 0, 0.33, 1, 3, and 5 hr. The reaction rates were determined from the initial rates and the results were reported using epichlorohydrin as a reference. 3,4-Epoxybutene alkylated NBP and deoxyguanosine at rates that were 31% and 14%, respectively, of the alkylation by epichlorohydrin. In agreement with Hemminki et al [1980], Citti et al [1984] published data suggesting that 3,4-epoxybutene alkylates deoxyguanosine and DNA in vitro. The main products formed from both deoxyguanosine and DNA were 7-(2-hydroxy-3-buten-1-yl)deoxyguanosine and 7-(1-hydroxy-3-buten-2-yl)deoxyguanosine.

Lawley and Brookes [1967] reported that diepoxybutane reacts with DNA in a manner typical of bifunctional alkylating agents and causes interstrand cross-linking in DNA. Salmon sperm DNA (2 mg/ml, 5.4 mM DNA phosphorus) was treated with redistilled diepoxybutane (2.4 mg/ml, 28 mM) at 37°C. Samples were withdrawn after 2, 5, 7, 24, 48, 72, 120, 168, and 193 hr. Ultraviolet spectroscopy was used to measure the reaction of diepoxybutane with DNA. At 37°C, diepoxybutane reacted with DNA slowly, as shown by changes in ultraviolet absorption of the reaction mixtures.

The extent of interstrand cross-linking (ie, covalent linkage of the two DNA strands by the reaction of diepoxybutane with a nucleotide base in each DNA strand) was studied by measuring the reversible denaturation (renaturation) of diepoxybutane-treated DNA. In this experiment, diepoxybutane-treated DNA was denatured at 60°C for various time periods and then rapidly cooled. Diepoxybutane-treated DNA renatured to a greater extent than did normal untreated DNA, suggeting that diepoxybutane-treated DNA was cross-linked by a diepoxybutane bridge covalently joining the two DNA strands.

In summary, the above three in vitro studies indicate that 3,4-epoxybutene and diepoxybutane can alkylate DNA and that diepoxybutane causes interstrand cross-

links in DNA. In addition, preliminary in vivo data suggest that 1,3-butadiene alkylates DNA in rats and mice after inhalation exposure [Laib et al, 1985], presumably via epoxide intermediates.

MUTAGENICITY OF 3,4-EPOXYBUTENE

In addition to studying the mutagenicity of 1,3-butadiene, de Meester et al [1978] tested its monoepoxide metabolite, 3,4-epoxybutene, by the plate incorporation method in *Salmonella*. Strains TA1537, TA1538, and TA98 exhibited a negative response. Reversion to histidine prototrophy was observed with strains TA1530, TA1535, and TA100, with maximal numbers of revertants occurring at a 3,4-epoxybutene concentration of 100 μmol/plate.

The mutagenic potential of 3,4-epoxybutene was studied in the fluctuation test with *Klebsiella pneumoniae* as the test organism [Voogd et al, 1981]. The genetic characteristic studied was streptomycin resistance. The average spontaneous mutation rate for streptomycin resistance was 0.1676×10^{-9}. Triplicate experiments were averaged, and the results were expressed as the quotient of the observed and spontaneous mutation rates. At 1 and 2 mM 3,4-epoxybutene, the quotients were 1.7 and 2.5, respectively, providing evidence of a dose-related positive response.

In addition to studying the alkylating activity of 3,4-epoxybutene, Hemminki et al [1980] studied its mutagenicity (reversion to tryptophan prototrophy) in *Escherichia coli* strain WP2 *uvrA*. The concentrations of 3,4-epoxybutene used were not specified but were based on toxicity determinations. Although the study suggests that 3,4-epoxybutene is mutagenic in *E coli* WP2 *uvrA*, there was no indication of a dose-related response; the result for only one dose was reported and that dose was unspecified. Although this study is of limited use for risk-assessment purposes, it supports the study of Voogd et al [1981] in suggesting that 3,4-epoxybutene is mutagenic in bacteria.

Unpublished data suggest that 3,4-epoxybutene injected intraperitoneally induces sister chromatid exchange (SCE) and chromosomal aberrations in mouse bone marrow cells (Dr. James W. Allen, personal communication). SCE involves the reciprocal exchange of DNA segments between sister chromatids and is considered an indication of DNA damage. A dose-related increase in SCEs was observed at 3,4-epoxybutene doses ranging from 10 mg/kg to 150 mg/kg. A dose-related increase in chromosomal aberrations at 25–150 mg/kg was also observed. The types of aberrations observed included chromatid and chromosome/isochromatid breaks, chromatid deletions, fragments, acentric fragments, and polyploidy. It is possible that the 3,4-epoxybutene was biotransformed after intraperitoneal injection and that the positive results for SCE and chromosomal aberrations in bone marrow cells were due to a metabolite of 3,4-epoxybutene.

In summary, 3,4-expoxybutene is mutagenic in bacteria and induces SCE and chromosomal aberrations in mice.

GENOTOXICITY OF 1,2:3,4-DIEPOXYBUTANE

Information on the genotoxicity of diepoxybutane can be found in a review on the genotoxicity of several epoxides by Ehrenberg and Hussain [1981]. This review covers much of the early literature on diepoxybutane that is not discussed below,

including testing in plants. This section on the genotoxicity of diepoxybutane covers more recent information and provides an indication of the types of genotoxic effects rather than an exhaustive survey of the literature.

Studies in Bacteria

Voogd et al [1981] investigated the mutagenic potential of diepoxybutane in *K pneumoniae* in the same paper cited previously for the mutagenicity of 3,4-epoxybutene. At an equal chemical concentration (1 mM), diepoxybutane was approximately 16 times as mutagenic as 3,4-epoxybutene. The quotients of observed and spontaneous rates of mutation to streptomycin resistance for 0.05, 0.1, 0.2, 0.5, and 1 mM diepoxybutane were 1.7, 3.1, 6.2, 15.7, and 27, respectively. These results clearly indicate that diepoxybutane is mutagenic in *K pneumoniae* and provide strong evidence of a dose-related response as well.

Diepoxybutane is also mutagenic in the *S typhimurium* histidine reversion assay [Wade et al, 1979]. Plate incorporation assays were performed with strains TA98 and TA100, and averages of two to five determinations were reported. At 0.02, 0.10, and 0.50 mg of diepoxybutane per plate, there were 196, 325, and 663 revertant colonies per plate with strain TA100 and 32, 22, and 29 revertant colonies per plate with strain TA98. These results suggest that diepoxybutane is a base-pair-substitution mutagen in *S typhimurium* because it produced a dose-related positive response in strain TA100. Although strain TA100 is not specific for base-pair-substitution mutagens, it responds well to such mutagens, and the result in strain TA98, which detects many frameshift mutagens, was negative.

Studies in Fungi

The mutagenic potential of diepoxybutane in the yeast *Saccharomyces cerevisiae* was studied by Olszewska and Kilbey [1975]. They used a diploid yeast strain that is homozygous for the *ilv* mutation and therefore requires isoleucine and valine to grow. Kinetic studies of the induction of revertants were carried out by treating cells for various times with 0.1 M diepoxybutane at 25°C. Diepoxybutane induced an increase in *ilv* reversions with increasing time of exposure up to 20 min. At 5, 10, 15, and 20 min, the number of ilv^+ revertants per 10^6 cells was about 3, 10, 20, and 38, respectively. Reversion of the *ilv* mutation indicates that diepoxybutane induces point mutations in yeast.

Zaborowska et al [1983] have shown that diepoxybutane induces mitotic crossing-over and mitotic gene conversion in the SBTD and D7 strains of *S cerevisiae*. Stationary-phase cells were treated with 0.4% (vol/vol) diepoxybutane at 30°C for 15, 30, and 45 min. The results of these experiments are shown in Table I. The frequency of mitotic crossing-over in the SBTD was dose (exposure time) related. Dose-dependence of mitotic crossing-over in strain D7 is less clear. Dose-related increases in mitotic gene conversion were obtained in both strains. Taken together, these results indicate that diepoxybutane is recombinogenic in yeast, and recombinogenicity is an indication of DNA damage.

Luker and Kilbey [1982] reported that diepoxybutane causes point mutations and multigenic deletions in *Neurospora crassa*. They developed a *Neurospora* heterokaryon in which both point mutations and deletions can be detected by the use of selective techniques. Point mutations were scored by reversion to adenine prototrophy. Deletions were detected by first assaying for resistance to *p*-fluorophenylalanine

TABLE I. Induction of Mitotic Gene Conversion and Mitotic Crossing-Over in SBTD and D7 Strains of *S cerevisiae* by Diepoxybutane*

Exposure to 0.4% diepoxybutane (min)	Survival	Mitotic crossovers (%)	Convertants[a]
SBTD strain			
0	100	0	0.7
15	100	1.4	86.6
30	93.2	2.8	167.1
45	41.3	6.1	515.2
D7 strain			
0	100	0	0
15	100	1.5	40.4
30	78.4	1.7	278.0

*Source: Zaborowska et al [1983].
[a]Convertants calculated per 10^7 survivors in the SBTD strain and per 10^6 survivors in the D7 strain.

TABLE II. Induction of Adenine Reversions and pFPA Resistance in *Neurospora crassa* by 0.1 M Diepoxybutane*

Treatment (min)	Survival (%)	Heterokaryotic conidia screened ($\times 10^6$) for		Number of mutants scored		Mutation frequency $\times 10^{-6}$	
		ad^+	pFPA resistant	ad^+	fpr^+	ad^+	fpr^r
0	100.0	15.64	4.17	5	2	0.32	0.48
10	78.1	6.90	1.38	22	12	3.19	8.70
20	60.4	5.54	1.13	34	21	6.03	18.58
30	40.8	2.53	0.51	53	21	20.95	41.18

*Source: Luker and Kilbey [1982].

TABLE III. Analysis of $pFPA^r$ Mutants as Putative Deletions*

Treatment with 0.1 M diepoxybutane (min)	No. of pFPA-resistant mutants tested	No. acquiring sensitivity to cycloheximide	Putative deletions (%)
10	12	5	42
15	7	2	29
20	41	10	24
30	94	21	22
45	25	10	40
60	11	2	18
Total	190	50	26.3

*Source: Luker and Kilbey [1982].

(pFPA) and then testing for sensitivity to cycloheximide. These two genes are closely linked on chromosome V. Suspensions of *Neurospora* conidia were treated for various times with 0.1 M diepoxybutane. Diepoxybutane induced dose (exposure time)-related increases in both adenine reversions and pFPA resistance, as shown in Table II. The results shown in Table III suggest that about one fourth of the pFPA-resistant

mutants were deletions rather than point mutations because pFPA resistance was associated with sensitivity to cycloheximide 26.3% of the time. The evidence therefore shows that diepoxybutane induces both point mutations and multigenic deletions in *Neurospora*.

Studies in Mammalian Cells

Dean and Hodson-Walker [1979] tested diepoxybutane for the induction of chromosomal aberrations in cultured rat liver epithelial-like cells. The appropriate concentrations of diepoxybutane for testing were determined from cytotoxicity studies. Because diepoxybutane is volatile, sealed flask cultures were used. The results (Table IV) suggest that diepoxybutane is clastogenic in rat liver cells, producing significant chromatid lesions at 0.1 μg/ml. At the higher concentrations, fewer metaphases were apparently available for cytogenetic analysis, perhaps because of cytotoxic effects at these doses.

Perry and Evans [1975] reported that exposure of cultured Chinese hamster ovary cells to diepoxybutane resulted in a dose-related induction in SCEs. The cells were treated with 10 μM bromodeoxyuridine and 0.3 μM or 3 μM diepoxybutane for two cycles of DNA replication. SCEs/cell at 0, 0.3, and 3 μM diepoxybutane were 12.2, 20.2, and 90.9, respectively.

In Vivo Studies

Studies of the mutagenic potential of diepoxybutane in whole animals have been carried out in mice and *Drosophila melanogaster*.

Conner et al [1983] studied the ability of diepoxybutane to induce in vivo SCE in bone marrow, alveolar macrophages, and regenerating liver cells in mice. The dose-response studies were performed at 10–291 μmol of diepoxybutane per kg (intraperitoneal injection) in three intact and three partially hepatectomized Swiss Webster mice per dose. Bone marrow and alveolar macrophage cells from intact mice and regenerating liver cells from hepatectomized mice were analyzed for SCE. As shown in Table V, diepoxybutane produced similar dose-dependent responses for SCE in bone marrow, alveolar macrophages, and regenerating liver cells. Conner et al [1983] also reported that the DNA lesions did not persist beyond one cell cycle. These results show that diepoxybutane is very effective in producing a dose-dependent SCE response, but that the initially induced lesions disappear in subsequent division cycles.

The sex-linked recessive lethal test in *Drosophila*, which tests the mutagenic potential of chemicals in the germ line of an intact animal, was positive for diepoxybutane in two studies from the same laboratory. In the first study, Sankaranarayanan [1983] exposed wild-type Berlin-K 3- or 4-day-old male flies to 2 mM diepoxybutane

TABLE IV. Effects of Diepoxybutane on Chromosomes of Rat Liver Cells*

Diepoxybutane (μg/ml)	No. of cells analyzed	Chromatid gaps (%)	Chromatid deletions (%)	Chromatid exchanges (%)	Chromosome aberrations (%)
0	269	2.6	0.4	0	0.4
0.1	117	11.1	4.3	8.5	0.9
0.5	24	8.3	20.8	33.3	0
1.0	29	0	0	3.4	0

*Source: Dean and Hodson-Walker [1979].

TABLE V. SCE Frequencies Induced in Bone Marrow, Alveolar Macrophages, and Regenerating Liver Cells of Swiss Webster Mice Following Injection of Diepoxybutane*

Diepoxybutane (μmol/kg)	Bone marrow		Alveolar macrophages		Regenerating liver	
	A[a]	B[b]	A	B	A	B
Hepatectomized mice						
0	4.2 ± 0.6	66	3.5 ± 0.6	77	3.7 ± 0.9	80
10	7.2 ± 0.9	63	7.9 ± 0.4	71	7.0 ± 1.4	73
39	8.1 ± 1.4	72	9.8 ± 1.0	70	11.9 ± 2.6	69
97	10.9 ± 1.5	63	13.4 ± 3.4	75	14.9 ± 4.0	69
193	22.3 ± 3.5	44	23.6 ± 4.5	59	28.9 ± 6.1	43
291	32.0 ± 6.6	22	30.4 ± 4.9	23	31.1 ± 5.9	29
Intact mice						
0	3.0 ± 0.8	64	3.6 ± 0.5	63		
10	5.4 ± 0.6	58	6.7 ± 1.2	54		
39	8.8 ± 0.8	63	10.2 ± 0.3	57		
97	9.7 ± 1.5	63	12.8 ± 2.3	62		
193	14.6 ± 2.1	43	17.1 ± 2.2	41		
291	27.3 ± 3.7	46	28.6 ± 3.8	42		

*Source: Conner et al [1983].

[a]Mean SCE/cell ± SD of three mice at each dose. Individual animal means were calculated from SCEs scored in 20 cells of each type.

[b]Mean number of second division cells observed in 100 consecutive metaphases.

in 5% sucrose by feeding for 48 hr. The males were mated to *Oster* females to raise three successive 2-day broods (A, B, and C), and the F_1 female progeny were used in the tests for lethals. Brood A tests mature spermatozoa, brood B tests late spermatids, and brood C tests early spermatids. The results are shown in Table VI. A concurrent no-exposure control was not done in this study or in the subsequent study described below. However, a historical control value for *Drosophila* of 0.18% has been established in the same laboratory from the evaluation of 13,151 chromosomes [Vogel, 1976]. The results suggests that diepoxybutane is a strong inducer of sex-linked recessive lethal mutations (6.5%, 4.8%, and 4.6% compared to the historical control value of 0.18%). The results also indicate that mature spermatozoa (brood A) respond with higher frequencies of recessive lethals than late and early spermatids (broods B and C).

Similar positive results (Table VI) were obtained in a later study in *Drosophila* [Sankaranarayanan et al, 1983]. The experimental details in this study were identical to those described above, except that *Canton-S* and *ebony* males were exposed to diepoxybutane and mated to *Muller-5* females. These results demonstrate that the strong mutagenic response is independent of the strains employed. The strongly positive results in the sex-linked recessive lethal test provide clear evidence that diepoxybutane reaches the gonads and is strongly mutagenic in germ cells of *Drosophila*.

There is evidence that diepoxybutane induces chromosome damage in germ cells of *Drosophila* [Zimmering, 1983]. Treated males carried an X chromosome in the form of a closed ring and a Y chromosome carrying dominant markers, one at the end of the long arm of the Y and one at the end of the short arm. The males were permitted to feed on a solution of 1.25 mM diepoxybutane in 5% sucrose for 24 hr and then mated with repair-proficient (ordinary) females or to repair-deficient fe-

TABLE VI. Frequencies of Sex-Linked Recessive Lethals Induced by 2 mM Diepoxybutane in Postmeiotic Male Germ Cells of *Drosophila*

Experimental strain	Brood[a]	No. of chromosomes	Lethals	
			No.	%
Berlin-K[b]	A	800	42	6.5
	B	914	32	4.8
	C	840	30	4.6
Canton-S[c]				
Experiment 1	A	934	88	9.4
	B	949	68	7.2
	C	960	66	6.9
Experiment 2	A	938	64	6.8
	B	951	54	5.7
	C	350	14	4.0
Ebony[c]				
Experiment 1	A	932	88	9.4
	B	912	65	7.1
	C	925	56	6.1
Experiment 2	A	876	57	6.5
	B	924	41	4.4
	C	885	33	3.7

[a]Brood A corresponds to treatment of mature spermatozoa; brood B corresponds to late spermatids; and brood C corresponds to early spermatids.
[b]Taken from Sankaranarayanan [1983].
[c]Taken from Sankaranarayanan et al [1983].

TABLE VII. Chromosome Loss in *Drosophila* From Matings of Males With Repair-Proficient (RP) and Repair-Deficient (RD) Females*,**

Series	Female	N	Complete loss	Partial loss	Percent induced	
					Complete loss	Partial loss
Control	RP	8,551	51	0		
Treated		7,390	515	8	6.37	0.11
Control	RD	3,178	30	2		
Treated		1,285	82	39	5.44	2.97

*All induced frequencies are statistically significant at or below the 0.01 level. The repair-deficient mutant was *mei-9*[a], which is deficient in excision repair.
**Source: Zimmering [1983]

males. There was no evidence of toxicity in the treated males. The F_1 offspring were scored for complete loss of the X or Y chromosomes (in ring-X males, virtually all complete loss is attributable to ring loss) and for partial loss of the Y chromosome, indicated by the loss of one but not both of the Y chromosome markers. Complete loss indicates chromosome breakage and/or SCE. Partial loss of the Y chromosome is a consequence of breakage. Results shown in Table VII provide evidence of a relatively strong effect on complete loss (5–6%) and a significant increase in partial loss which is most apparent from matings with the repair-deficient females (approx 3%).

In summary, the results of the *Drosophila* experiments assaying for sex-linked recessive lethals and chromosome loss provide strong evidence that diepoxybutane is a mutagen and a chromosome-damaging agent in germ cells of *Drosophila*. In

addition, the results of the SCE assay in mice suggest that diepoxybutane is a DNA-damaging agent in mice. The diepoxybutane was injected intraperitoneally in the mice and fed to the *Drosophila*. It is possible that the diepoxybutane was biotransformed before reaching mouse bone marrow or *Drosophila* gonads. Therefore, the mutagenic, clastogenic, or DNA-damaging effects described in these studies may be due to a metabolite of diepoxybutane.

MUTAGENICITY OF 4-VINYLCYCLOHEXENE AND ITS METABOLITES

Under certain conditions, such as during rubber curing (vulcanization), 1,3-butadiene can dimerize to form 4-vinylcyclohexene [Rappaport and Fraser, 1976]. Unpublished data from the National Toxicology Program [1985] indicate that 4-vinylcyclohexene was not mutagenic in the *Salmonella* preincubation assay (strain TA100, TA1535, TA1537, and TA98) in the presence or absence of liver S9 mix prepared from chemically induced rats or hamsters.

In contrast to the negative mutagenicity response of 4-vinylcyclohexene, its potential mono- and diepoxide metabolites (including 4-vinyl-1,2-epoxycyclohexane, 4-epoxyethyl-1,2-dihydrocyclohexane, and 4-vinylcyclohexene diepoxide) are mutagenic or clastogenic in various in vitro prokaryotic and eukaryotic test systems [Murray and Cummins, 1979; Simmon and Baden, 1980; Turchi et al, 1981; Voogd et al, 1981]. These compounds are base-pair-substitution mutagens, in agreement with the data for 1,3-butadiene and its potential epoxide metabolites.

SUMMARY AND CONCLUSIONS

The available information on the mutagenicity of 1,3-butadiene is quite limited in that only three studies have been reported. All three studies, however, indicate that 1,3-butadiene is a mutagen in *S typhimurium*. The weight of the available evidence suggests that 1,3-butadiene is mutagenic only in the presence of a liver S9 metabolic activation system. No whole animal studies have been reported. These results suggest that 1,3-butadiene is a promutagen in bacteria (ie, its mutagenicity depends on metabolic activation).

In vitro data suggest that 1,3-butadiene is metabolized to 3,4-epoxybutene and then to diepoxybutane. Preliminary evidence in rats suggests that 1,3-butadiene is metabolized to 3,4-epoxybutene in vivo, indicating that the metabolic pathway outlined on the basis of in vitro data may occur in vivo.

There is no information on the metabolism of 1,3-butadiene in humans. However, a scientific basis for the extrapolation from animal metabolism data to humans for 1,3-butadiene is provided by the similarities in the epoxidation of the isolated double bond in benzo(a)pyrene by organ and tissue cultures from animal and human sources [Autrup et al, 1980].

The epoxide intermediate 3,4-epoxybutene is a monofunctional alkylating agent, is a direct-acting mutagen in bacteria (*S typhimurium*, *K pneumoniae*, and *E coli*), and induces SCE and chromosomal aberrations in mice. Diepoxybutane is a bifunctional alkylating agent, as such it can form cross-links between the two strands of DNA. It is mutagenic in bacteria (*K pneumoniae* and *S typhimurium*), fungi (yeast and *Neurospora*), and the germ cells of *Drosophila*. It also induces DNA damage in cultured hamster cells and in mice, is clastogenic in fungi and cultured rat cells, and

produces chromosome damage/breakage in *Drosophila* germ cells. Therefore, the evidence indicates that 3,4-epoxybutene and diepoxybutane are mutagens/clastogens in microbes and animals.

Exposure of rodents to 1,3-butadiene results in ovarian tumors and gonadal atrophy in mice [Huff et al, 1985] and testicular tumors in rats [Hazleton Laboratories Europe, Ltd., 1981], which offers suggestive evidence that 1,3-butadiene (or. more likely, a metabolite of 1,3-butadiene) may reach the germ cells. There is also evidence that the dimer of 1,3-butadiene, 4-vinylcyclohexene, causes ovarian tumors in mice [NTP, 1985].

The total body of evidence from the metabolism, mutagenicity, and carcinogenicity data suggests that 1,3-butadiene may present a genetic risk to humans. However, mutagenicity studies in mammalian test systems, as outlined in the US Environmental Protection Agency's *Proposed Guidelines for Mutagenicity Risk Assessment* [1984], should be conducted to further characterize the mutagenic potential of 1,3-butadiene.

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